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Reproductive performance of *Helix pomatia* (Gastropoda: Pulmonata: Helicidae) and survival of its hatchlings under farm conditions

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Abstract: The reproductive ability of 1254 breeding individuals of *Helix pomatia* originating from a local wild population were studied. Reproduction was carried out in a greenhouse at a stocking density of 51.2 breeding snails per m^2. The reproductive season was 83 days long. From 30 May to 21 August 2003 almost all the snails laid eggs at least one time, but 25.1% of the snails laid eggs twice, and 5.2% laid eggs three times. The mean number of eggs per clutch for all the breeding snails was 41.7. Because of the multiple laying of eggs, the number of eggs laid in the 2003 season averaged 61.5 per breeding snail, and the eggs' total biomass constituted 38.5% of the biomass of all breeding individuals. A significant ($P < 0.05$) increase in egg laying was found from 30 May to 21 July. This period was followed by a rapid decline in reproductive intensity, until egg laying ceased on 21 August. Breeding snails laid 77100 eggs, out of which 40000 eggs hatched. From the hatched eggs 27000 two-to-three-week-old hatchlings were obtained. Of the obtained hatchlings 15000 were released into a greenhouse; 32.0% of these individuals survived winter hibernation in a pen. In May of the next year, the 4800 hatchlings were released into a field pen, at a density of 260 specimens per m^2. They reached a mean body mass of 19.1 g and mean shell diameter of 30.1 mm in July. The rapid rate of growth observed under farm conditions allows us to propose a two-year farming cycle for this species, from hatching to the stage of sexual maturity.

Key words: snail breeding, snail growing, snail protection, snail farming

In Poland there are still abundant live natural populations of the Roman snail (*Helix pomatia* Linneus 1758) (Stępczak 1976, Dyduch-Falniowska *et al.* 2001a, 2001b). As it has already happened with *Helix aspersa aspersa* (Müller 1774) and *Helix aspersa maxima* (Taylor 1883), it is likely that specimen numbers in these populations will be in danger of being reduced because of increasing exploitation by exporters for snail meat to other European markets (Łysak 1999). However, over the last 20 years, development of intensive farm-rearing of *Helix aspersa* has been developed, made possible by the snail's high fertility and the recognition of physiological factors and farming technology (Lucarz 1984, Daguzan 1989, Gomot and Gomot 1989, Gomot *et al.* 1989, Gomot and Deray 1990, Lazaridou-Dimitriadou and Bailey 1991). Due to its slower rate of growth, lower fertility, and difficulty with early spring hatching, *Helix pomatia* is regarded as a difficult species to breed compared to *Helix aspersa*. The development of technology for farm-rearing of this species has therefore become not only an economical concern but also an essential matter for environmental protection. Studies on the physiology of the reproductive biology of this species (Jeppensen 1976, Łysak *et al.* 2002) provide the basic background for its intensive cultivation. The aim of the present study was to develop the technology to farm the Roman snail, based on its life cycle and to enhance its rate of reproduction.

MATERIAL AND METHODS

On 25 April 2003, a group of 1254 adult Roman snails, *Helix pomatia*, were harvested from a natural population in a park surrounding the Radziwill family residence in Balice, now belonging to the National Research Institute of Animal Production in Kraków (Poland). Snails were placed in a pen in an unheated greenhouse planted with white clover and grass. A hardened and turned-out aperture lip was used as a sign of sexual maturity. The mean body mass of full-grown snails was 21.9 g (SD 3.79, range from 13.88 g to 34.09 g) and the shell diameter was of 34.07 mm (SD 1.77, range from 29.1 mm to 39.2 mm).

Stocking density was 51.2 snails per m^2. Inside the pen, vegetation-free strips of land were left to facilitate the observation of snails laying eggs. In the pen, feed was placed on wooden pallets. A sprinkling system spread water each morning and afternoon. Snails were given extruded vegetable-mineral feed designed for breeding individuals of *Helix aspersa* and produced by the Farming Cooperative in Łubnica (Wielkopolska Province, Poland). The feed was 16.0% soya-bean protein and 12.4% calcium (Ca) in the form of chalk. A detailed composition of the feed was reserved by the manufacturer. Egg laying was monitored every morning. Observations were made on breeding snails that worked their way into the soil to lay eggs. First clutches were laid on 30 May, that is, 35 days after the snails were placed into the greenhouse pen. The last clutches were laid on 21 August. To prevent their escape, laying snails were covered with upturned flower pots. The next day, after egg laying was completed, egg clutches were collected from their hole in the soil. Egg clutches were incubated in soil in plastic trays. Approximately 15000 two-week-old hatchlings were released into a greenhouse pen with a density of 600 specimens per m^2 and fed until late autumn. During the winter, hatchlings hibernated in an unheated greenhouse pen covered with Styrofoam and a gardening fabric used to protect crops from ground frost. In mid-March of the following year, the hatchlings became active and were given snail feed. In mid-April, the protective fabrics were removed to insert wooden feeders. Then, when the spring frosts passed in mid-May, the 4,800 young snails were transferred from the greenhouse to a field pen with a density of 260 specimens per m^2.

Breeding snails that laid eggs were weighed, their shell diameters were measured, and numbers were painted on their shells. Snails were marked to permit further observations of additional egg-laying by marked specimens in the same season. Egg clutches from the marked breeding snails were weighed and egg numbers were counted. From the mass of the clutch and the number of eggs in the clutch, the mean mass of an egg was calculated. These data were used to determine the values of specific reproductive parameters. The increase of body mass and shell diameter of growing snails was meassured in September 2003, and May and July 2004. Each time, random samples of 150 specimens were collected for measurements. Temperature and relative humidity of the air in the greenhouse were measured 20 cm above the surface of the pen. Measurements were taken on workdays at 7:00 and 14:00 hours. The results of reproduction were analyzed using analysis of variance and one-way regression using Statgraphics software.

RESULTS

Microclimatic conditions in the greenhouse pen

Mean air temperature at 7:00 hours decreased from 20.1°C in June to 18.4°C in August, and the temperature at 14 hours ranged from 26.6°C in July to 30.0°C in August. On some days, the afternoon temperature reached 34.3°C in June and 35.9°C in August. There were no significant differences between all mean morning, afternoon, and mean of day monthly temperatures, respectively. Mean humidity of the air in the morning ranged from 77.9% in June to 86.3% in July. In the afternoon, the mean humidity of the air was 65.7% in July, but was only 54.9% and 47.2% in June and August, respectively. There were significant differences ($P < 0.05$) between each mean morning monthly humidity, and highly significant differences ($P < 0.01$) between all mean afternoon and mean daily humidity, respectively. Snails in the greenhouse were active at least until midday, copulating and laying eggs.

Snails were raised under conditions of the natural photoperiod. The natural daylight in June lasted for 16.5-17.0 hours, during July it decreased from 16.5 hours to 15.5, and in August it decreased from 15.5 to 14.0 hours. On 21 August, when the last egg clutches of the season were found, the day length was 14 hours.

Reproductive parameters in the reproductive season

Significantly more snails laid eggs in the period of June-July than in August ($P < 0.01$), when reproductive intensity dropped by 65.7%. (Table 1). An increase in egg laying was found from the start of the reproductive season (30 May) to 21 July. This period was followed by a rapid decline in reproductive intensity, until egg laying ceased on 21 August. In June, the mean number of clutches and eggs laid during 24 hours per m^2 was almost the same as July, while in August this parameter dropped significantly ($P < 0.05$) by 65.5% and 76.2%, respectively. Between June and August, the number of eggs per body mass of parent decreased very significantly ($P < 0.01$) by 38.4%. In successive months, the mean number of eggs per clutch decreased rapidly, and in August the mean number of eggs per clutch was 41.6% significantly lower ($P < 0.01$) than in June. Mean mass per one egg decreased significantly ($P < 0.01$) between June and the period of July-August by 5.0%. The mean mass of clutches declined very significantly ($P < 0.01$) between June, July, and August, by 44.7% during the whole period. The relative mass of a clutch, expressed as a percentage of parental mass, also decreased significantly ($P < 0.01$) by 40.5%.

Significant ($P < 0.05$) and highly significant ($P < 0.01$) negative correlation coefficients were found for the relationship between the egg-laying intensity per 1 day per 1 m^2, and the average body mass and shell diameter of Roman snails laying eggs on a particular day (Table 2). Positive, highly significant correlations were found for the relationship between the individual body mass of a snail and the mean egg mass in the clutch. The same correlation was found for the diameter of the shell in relation to the average mass of a single egg and the mass of a clutch.

Multiple egg laying by the snails during the same reproductive season

Some snails began laying a second clutch of eggs by the middle of June and some began to lay eggs for the third time in late June and in July. In August, only eggs laid by snails laying for the third time in that season were found. During the three months of observation, 25.1% snails laid eggs twice and 5.2% laid three times. The mean interval between the first and the second clutches was 21.7 days; the mean interval

Table 1. Reproduction of *Helix pomatia* housed in a greenhouse pen.

Parameter	Month	Mean	SE	SD	Range
Mean percent of individuals laying eggs per 24 h	June	1.99^{B}	8.61	1.74	0.16-6.06
	July	2.52^{B}	7.85	1.19	0.64-4.78
	August	0.79^{A}	5.48	0.39	0.24-1.36
Mean number of clutches laid per 24 h per m^2 of pen surface area; stocking density was 51.2 snails per m^2	June	1.02^{b}	0.22	0.89	0.08-3.10
	July	1.29^{b}	0.19	0.61	0.33-2.45
	August	0.40^{a}	0.08	0.20	0.12-0.69
Mean number of eggs laid per 24 h per m^2 of pen surface area; stocking density was 51.2 snails per m^2	June	48.81^{b}	11.62	46.48	3.42-163.61
	July	46.52^{b}	8.49	26.85	9.91-102.90
	August	11.33^{a}	2.29	6.62	3.10-19.64
Mean number of eggs laid per 1 g of body mass	June	2.29^{C}	0.05	0.81	0.56-6.11
	July	1.78^{B}	0.06	0.82	0.61-5.63
	August	1.41^{A}	0.07	0.46	0.26-2.40
Mean number of eggs per clutch	June	48.1^{C}	0.88	14.82	14-89
	July	35.9^{B}	1.05	15.47	11-88
	August	28.9^{A}	1.45	10.46	5-67
Mean mass of one egg in clutch (mg)	June	138.7^{B}	1.33	22.11	84-226
	July	133.1^{A}	1.38	19.39	92-200
	August	130.3^{A}	3.33	22.78	85-179
Mean mass per clutch (g)	June	6.57^{C}	0.12	2.07	2.23-13.60
	July	4.72^{B}	0.13	1.93	1.72-13.02
	August	3.63^{A}	0.18	1.20	0.71-6.20
Mean mass of clutch per mass of parent snail (%)	June	30.37^{C}	0.57	9.60	8.09-60.11
	July	23.26^{B}	0.65	9.11	9.28-52.23
	August	18.05^{A}	0.81	5.50	6.11-28.19

a, b, c = significant differences ($P < 0.05$).
A, B, C = highly significant differences ($P < 0.01$).

between the second and the third clutches was 23.2 days. The mean total number of eggs from the three clutches was 117 (range 84-184). All reproductive parameters were higher for the first clutch than for the second. For the mean mass of clutch, differences from first to second and third clutch were highly significant ($P < 0.01$). The same highly significant differences ($P < 0.01$) were observed for mean percentage mass of clutch per mass of parent, and for the number of eggs per g mass of clutch (Table 3).

Reproduction for the entire period of observation

In the entire reproduction season, the mean number of eggs per clutch was 41.7, but the mean number of eggs laid during the entire reproductive period of 3 months was 61.5 per reproductive snail, because 30.3% of the snails laid eggs two or three times during the season. The mean mass of a clutch was 5.6 g, the mean mass of one egg was 132.1 mg, and the mean number of eggs per 1 g of snail biomass was 2.0. Total egg biomass was 38.5% of the total mass of the reproductive adults. In total, the observed snails laid in total 77100 eggs over 83 days, yielding 3145.5 eggs per m^2 of pen.

Rearing performance

Breeding snails laid 77100 eggs, out of which 40000 eggs were hatched. From the hatched eggs 27000 two-to-three-week-old hatchlings were obtained. Of the obtained hatchlings 15000 were released into the greenhouse, from which 7823 specimens or 52.2% of the initial population survived until 15 September.

A total of 4864 individuals released into the greenhouse, 32.4% of the initial population, survived winter hibernation. In May of the next year, the 4800 hatchlings were released into a field pen with a density of 260 individuals per m^2. By September, the mean diameter of the shells of these snails had reached 14.2 mm and the body mass reached 2.5 g. After the winter hibernation, by July 2004, the mean diameter of the shells had reached the minimum of 30.1 mm required in Poland for commercial snails to be collected from natural

Table 2. Results of one-way regression analysis for egg-laying traits of *Helix pomatia*, 1 June to 21 July 2003.

Parameter I	Parameter II	Correlation coefficient (r)	Significance
Number of clutches per m^2 of pen area per day, from 30 May to 21 August	Mean mass of egg-laying snails	−0.68	$P < 0.05$
	Mean diameter of parent shell	−0.71	$P < 0.01$
	Mean mass of egg	−0.71	$P < 0.01$
Number of eggs per m^2 of pen area per day, from 30 May to 21 August	Mean mass of egg-laying snails	−0.67	$P < 0.05$
	Mean diameter of parent shell	−0.67	$P < 0.05$
	Mean mass of one egg	−0.73	$P < 0.05$
Mass of clutch, from 30 May to 21 August	Number of eggs per clutch	0.87	$P < 0.01$
Mass of egg-laying snails	Mass of one egg	0.44	$P < 0.01$
	Mass of clutch	—	not significant
	Number of eggs per clutch	—	not significant
Diameter of parent shell	Mass of one egg	0.48	$P < 0.01$
	Mass of clutch	0.39	$P < 0.01$
	Number of eggs per clutch	—	not significant

Table 3. Reproductive performance of *Helix pomatia* snails which laid eggs 3 times per season.

Clutch number	Mean number of egg per clutch	Mean mass of clutch (g)	Mean mass of clutch per mass of the parent snail (%)	Mean mass of one egg in clutch (mg)	Number of eggs laid per 1 g of body mass
I	49.6	6.8^{A}	32.5^{A}	138.6	2.4^{A}
II	35.2	4.5^{B}	22.2^{B}	131.0	1.7^{B}
III	32.1	4.0^{B}	19.2^{B}	125.0	1.6^{B}

A, B = highly significant differences ($P < 0.01$).

populations. Also at that date, the mean body mass increased to 19.1 g (Figure 1). In April 2004, the coefficients of variation for the body mass and shell diameter were high (80.0% and 26.4%, respectively), due to the two-month differences in age of the hatchlings, which hatched from June to August of the previous year. In July, the coefficients of variation decreased to 33.3% and 11.8%, respectively, because the snails grew.

DISCUSSION

Microclimatic conditions

Gomot (1990) found that under laboratory conditions, Roman snails have the highest reproductive activity when photoperiods last 18 hours. He suggested a the temperature of 20°C partly compensates for the effect of shorter photoperiods on reproduction. A similar effect of higher temperature at shorter photoperiods was found by Jess and Marks (1998) for *Helix aspersa maxima*, and by Gomot *et al.* (1989) for *Helix aspersa*. Similarly, we suspect that greenhouse temperatures in the mornings of June-July 2003, which averaged approximately 20°C compensated for the influence of reduced daylight. In August, however, the natural photoperiod declined below 15 hours, which seemed to cause a rapid decrease in reproductive intensity and termination of egg-laying by snails, despite of the still-high air temperature. Another potential cause of sustained high reproductive intensity in July was the statistically significant higher relative humidity in June than in August. Relative humidity in June was close to the optimal humidity of 75-85% recommended for breading in *Helix aspersa*.

Reproductive parameters in the reproductive season

Roman snails maintained in pens in the field in Poland during June-August 2004 peaked in their reproductive output in June (Chmielewski 2005). The greenhouse pen used in the current study probably provided better microclimatic and feeding conditions for reproduction than was possible in the field pen, and permitted the reproductive intensity in July to remain at the same high level as in June. The reproductive output of the snails in the current greenhouse study

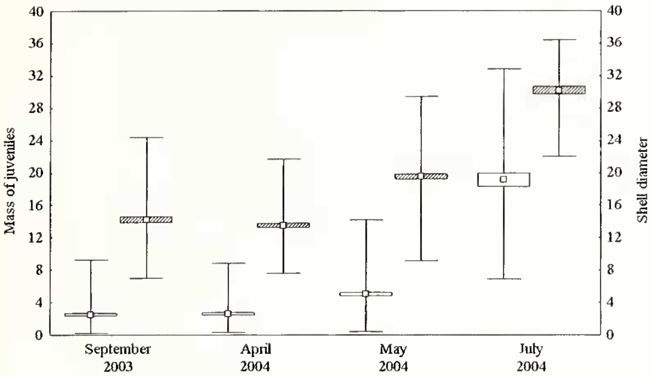

Figure 1. Mean masses (open boxes, in g) and shell diameters (hatched boxes, in mm) of juveniles of *Helix pomatia* hatching from eggs laid in the greenhouse pen in 2003. Box represents ± standard error around mean; whiskers indicate maximum and minimum values.

is similar to that for the month of June of snails maintained under natural conditions (Łysak *et al.* 2001).

Multiple egg laying by the snails during the same reproductive season

In the current study, some individuals laid second and third clutches of eggs in July and in August. Gomot (1990) found that under experimental conditions the frequency of egg-laying by the same snails was higher during a consistent 18-hour photoperiod than during a shorter 8-hour one. Therefore, the repetitive laying of eggs may be a function of both the photoperiod as well as the length of the reproductive season. The first clutches of eggs laid by Roman snails in the season were significantly heavier ($P < 0.01$) and proportionally greater in relation to parental body mass. Clutches of other *Helix* species living in natural conditions and laid at the beginning of the reproductive season also contain more eggs than those laid later in the season (Lazaridou-Dimitriadou and Bailey 1991). However, the clutches of eggs laid later in the season may have been the second and third clutches of the same snails, which may explain why they contained fewer eggs.

Reproduction for the entire period of observation and rearing performance

Our results from rearing older hatchlings, before and after hibernation in the winter of 2003/2004 in a greenhouse pen, and later moving them to a field pen, provides a basis for developing a technology for farming Roman snails over a two-year cycle. Over half of the specimens survived until the time of winter hibernation from the group of two-to-three-week old Roman snails raised in the greenhouse pen 2003, which correspondes to the survival rate required by the technologies for the raising of *Helix aspersa* in its first year of life. We obtained a satisfactory survival rate of 32.0% for the Roman snail hatchlings, calculated from the moment they were placed in the greenhouse pen in the summer of 2003 to the moment they were moved to the field pen in May 2004. The survival rate through July 2004 was lower than the 50% specified by breeding techniques for *Helix aspersa*, but the breeding cycle of the Roman snail is one season longer than that of *Helix aspersa* and it is separated by a period of winter torpor that is physiologically difficult for juvenile snails. The rapid rate of growth observed under farm conditions leads us to propose a two-year farming cycle for this species, from hatching to maturity.

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